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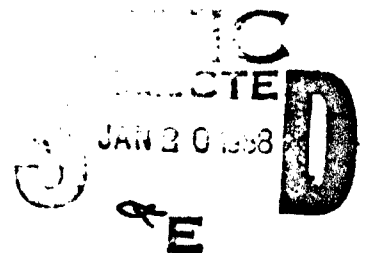
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ACUTE RESPIRATORY TRACT TOXICITY OF THE
TRICHOTHECENE MYCOTOXIN, T-2 TOXIN

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I. INTRODUCTION

The systemic toxicology of trichothecene mycotoxins in a variety of laboratory and farm animals is well documented. Toxicity reports following respiratory exposure, however, are mostly anecdotal. For instance, Ismailson first called attention to toxicosis secondary to mold spore inhalation in a binder twine factory. More recently, Forgacs² described symptoms of a severe pharyngitis, or burning sensation in the nose accompanied by bloody nasal discharge and a moderate to severe cough⁴ in workers that developed stachybotryotoxicosis after inhaling dusts from Stachybotrys-contaminated straw. Ueno³ reported 100% lethality of five mice 5 days after a 160-minute exposure to 140 ppb of T-2 mycotoxin. However, no information on aerosol generation or aerosol characterization was given, and only very limited information on exposure conditions was presented.

A report¹ issued in 1983 by the Committee on Protection Against Trichothecene Mycotoxins noted that there was a lack of information on inhalation exposure to trichothecene toxins and recommended that this route of exposure be studied. As a result of this committee's report, financial support for inhalation exposures to trichothecene toxins was developed, and studies of animals exposed to trichothecene aerosols were begun in 1984 in a limited number of laboratories. As a consequence of the recent initiation of these studies, most of the data presented in this report are preliminary in nature and subject to future modification.

II. BACKGROUND

Inhalation exposure to toxins could result in both local respiratory tract lesions as well as systemic toxicosis with all the concomitant

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lesions associated with either oral or parenteral administration. Toxins deposited in the respiratory tract could exert local toxic effects and/or be absorbed directly into the circulatory system, to be distributed to other organs and tissues. Additionally, a portion of the toxins, especially those deposited in the upper respiratory tract, may be cleared via the mucociliary mechanism from the respiratory tract to the pharynx where they could be swallowed. This would result in gastrointestinal exposure and absorption. The rate and principle mechanisms of toxin clearance from the respiratory tract are dependent upon the physical and chemical characteristics of the inhaled toxin. These factors result in exposure of other organs and tissues at varying dose rates.

Although the entire respiratory tract is exposed to inhaled toxins, the major subdivisions of the respiratory tract differ in size, cellular structure, function, and response to inhaled material. These factors and others cause the fate of inhaled particles to depend on their own size, shape, and solubility characteristics as well as the site of deposition.⁵ Aerodynamically large particles are deposited on the mucosae of the nasal passages and/or respiratory tree and may subsequently reach the gastrointestinal tract via ciliary mucus transport or be directly absorbed into the circulation.⁶⁻⁸ Whether particles are cleared from the upper respiratory tract by mucus transport or are absorbed depends primarily on the solubility of the particle, and both transport and absorption mechanisms may occur simultaneously. Small particles (i.e., $< 1.0 \mu\text{m}$ mass median aerodynamic diameter [MMAD]) are likely to penetrate to the alveoli where, if they are soluble in that environment, they may pass rapidly through the

alveolar wall into the pulmonary circulation; or, if insoluble, they may be ingested by macrophages. Particles ingested by alveolar macrophages are usually cleared from the respiratory tract by migration of these cells either into the pulmonary lymphatic system or into the bronchioles. The macrophages that have entered bronchioles may then travel via mucus transport to the tracheobronchial region, from which they will eventually be expelled and swallowed.

III. TRICOTHECENE CHARACTERISTICS

Many of the trichothecenes are sufficiently lipid-soluble⁴ that they would be expected to be cleared rapidly from the respiratory tract via the pulmonary circulation and subsequently induce systemic toxicosis. Nevertheless, data from experiments assessing trichothecene toxicity after dermal application indicate that most trichothecenes produce severe irritation at the application site. Thus, one would expect both local respiratory tract lesions and systemic toxicosis after respiratory tract exposure. Experiments from which the following reports are based were designed with the expectation that inhalation of most trichothecenes would produce a local respiratory lesion as well as systemic toxicosis. For expediency, T-2 toxin was selected as being representative of the other trichothecene mycotoxins.

IV. INTRATRACHEAL INSTILLATION AND INHALATION TOXICITY STUDIES OF T-2 TOXIN IN RATS AND MICE

A. Exposure Rationale, Protocols, and Clinical Effects

Initial studies in one author's laboratory (DAC) were designed to measure qualitatively the acute pulmonary toxicity of dissolved and undissolved T-2 toxin in rodents. The question to be answered was whether T-2 in solution, which was expected to pass rapidly into the

pulmonary circulation, was sufficiently active as a local irritant to produce respiratory distress or whether undissolved T-2 (as a saline suspension), with its longer contact time with respiratory tract tissue, was needed to produce local pulmonary inflammation. Intratracheal instillation rather than inhalation was selected as the initial method of choice for T-2 administration because of the ease of application and the assurance of accurate and reproducible delivery of T-2 toxin to the respiratory tract tissue. Consequently, dose-response curves were developed for T-2 dissolved in 5% ethanol, in 5% dimethylsulfoxide, and for a saline suspension of crystalline T-2 toxin, instilled intratracheally into both mice and rats. The results showed that a saline suspension of T-2 toxin administered intratracheally was at least 20 times more toxic to mice and rats than a solution of T-2 toxin administered intratracheally or intravenously (Creasia, unpublished data). In mice, the 24-hour LD₅₀ for a saline suspension of T-2 was 0.16 ± 0.06 mg/kg of body weight, while that for T-2 toxin dissolved in either ethanol or dimethylsulfoxide was 4.6 ± 0.3 mg/kg. In rats, the 24-hour LD₅₀ was 0.1 ± 0.1 mg/kg for a saline suspension of T-2 toxin and 2.2 ± 0.2 mg/kg for T-2 toxin dissolved in either 5% ethanol or 5% dimethylsulfoxide. Further analysis of the data also showed that at doses equal to or greater than the LD₅₀, T-2 administered as a suspension killed both mice and rats within 1 to 2 hours, while T-2 administered as a solution in either 5% ethanol or 5% dimethylsulfoxide, even at the approximate LD₁₀₀ dose level, did not kill either mice or rats in less than 15 hours (Creasia, unpublished data). As an explanation for this data, we presumed that the physical state of T-2 in solution allowed it to cross the alveolar epithelium rapidly without

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producing local lesions, enter the pulmonary circulation, and distribute to other organs and tissues, producing deaths after 15 hours from systemic toxicosis.⁹⁻¹¹ Conversely, it seems probable that the crystalline form of the T-2 in the suspension did not permit the rapid translocation of the toxin from the alveoli to the pulmonary circulation but allowed for longer contact between toxin and pulmonary epithelia. This prolonged contact conceivably produced local pulmonary lesions and respiratory distress with deaths within 1 or 2 hours.

In subsequent studies, Martin¹² found a decrease in the arterial partial pressure of oxygen and an increase in the partial pressure of carbon dioxide and decreased pH values in rats after intratracheal instillation, but not after intravenous injection of a saline suspension of T-2 toxin at 1.0 mg/kg of body weight (~ i.v. LD₉₉). Experiments designed to assess whether pulmonary edema was a possible cause for the altered blood gases failed to show any significant increase in lung fluids after either an LD₅₀ or an LD₁₀₀ dose of T-2 toxin (Creasia, unpublished data). Examination by light microscopy has also failed to demonstrate any significant alterations in pulmonary tissues after respiratory tract exposure to T-2 toxin that could account for the altered blood gases (Thurman and Creasia, 1984, unpublished data). In more recent studies (Templeton and Creasia, unpublished data) we were not able to confirm Martin's data.

The acute pulmonary toxicity data derived from intratracheal instillation of T-2 toxin indicated that an acute exposure to aerosols of the toxin could produce a definite health hazard to those so exposed. These data, together with some basic calculations, suggested that it would be feasible to generate T-2 toxin aerosols with a

sufficient mass concentration to produce measurable toxicity after a 10-minute aerosol exposure. Therefore, a series of experiments with mice and rats were designed to measure the acute toxicity of a 10-minute, nose-only exposure to an aerosol of T-2 toxin in either an ethanol solution or a saline suspension. The concentration-response data from these experiments indicated that the 24-hour LC₅₀ was 80 ± 20 $\mu\text{g/L}$ for mice exposed to an aerosol of T-2 toxin in an ethanol solution and 30 ± 8 μg for mice exposed to an aerosol of T-2 in a saline suspension (Creasia, unpublished data). When mice were exposed to aerosols of T-2 in solution, most of the animals exposed to higher aerosol concentrations (i.e., greater than the 24-hour LC₅₀) died within a few hours postexposure, and all died within 15 hours (Creasia, unpublished data). Immediately postexposure, all of the mice were lethargic and exhibited no grooming behavior, and most were prostrate. At aerosol mass concentrations below the 24-hour LC₅₀, the number of acute deaths decreased, as one would expect; however, animals that survived for 24 hours did not recover from overt toxic effects (lethargy, rough hair coat, tremors, etc.) during the study, and deaths among these mice continued for at least a week. Mice exposed to the lowest lethal aerosol concentrations (i.e., approximate 24-hour LC_{L0}) appeared relatively normal immediately postexposure. However, by 15 hours postexposure a few mice developed tremors, became lethargic, and then died before 48 hours. These data are interpreted as indicating that, at higher aerosol mass concentrations, T-2 toxin produced respiratory distress resulting in early lethality, while at lower aerosol concentrations, deaths occurred later due to a redistribution of the toxin from the lungs, with the associated systemic toxicosis.

A similar response, although with a lower 24-hour LC₅₀, was observed for mice exposed over 10 minutes to an aerosol of a T-2 toxin suspension (Creasia, unpublished data). At aerosol concentrations above the 24-hour LC₅₀, mice tended to die early (i.e., < 10 hours) and at lower aerosol concentrations at later time points (i.e., > 24 hours).

B. Lesions

Lesions observed via light microscopy were similar in mice regardless of whether the animals were exposed to: (1) an aerosol of a T-2 toxin suspension; (2) an aerosol of a T-2 toxin solution; (3) an intratracheally instilled T-2 toxin suspension or solution; or (4) an intravenously administered T-2 toxin solution. Surprisingly, no significant lesions were observed by light microscopy in either the upper respiratory tract or lungs in any of the mice exposed to either aerosol of T-2 toxin (Thurman and Creasia, 1984, unpublished data). Although still preliminary, these histopathologic results tend to support those previously observed when T-2 toxin was administered by intratracheal instillation. In mice examined 24 hours after aerosol exposure to all but the lowest mass concentration of toxin, lesions were characteristic of those routinely observed after systemic administration of acutely toxic doses of T-2 toxin.^{13,14} Lymphocytes within the cortex of the thymus were the most sensitive cells to the effects of the mycotoxin. Necrosis of cells in the red and white pulp of the spleen was routinely present but was quantitatively less than that seen in the thymic cortex. Necrosis of crypt epithelium in both the large and small intestine was present in mice exposed to aerosol mass concentrations greater than 150 µg T-2/L of air. Higher exposure concentrations were required to produce intestinal crypt epithelial necrosis than were

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required to produce the thymic or splenic lesions. In addition, degeneration and necrosis of parenchymal cells in the outermost portion of the zona fasciculata of the adrenal glands were present in some mice of the high-aerosol-mass concentration group. This circumferential band of damaged cells was multifocal or diffuse and only one to two layers wide.¹⁵

C. Toxicity as a Function of Dose Retention

As mentioned above, the responses of mice exposed for 10 minutes to an aerosol of T-2 generated from either a solution or suspension were similar, including the earlier deaths at higher concentrations, except that the dose-response curve was shifted to the left (i.e., the mice were more severely affected) when the animals were exposed to an aerosol of the T-2 suspension. While the early deaths observed after intratracheal instillation of T-2 were attributed to acute respiratory distress induced by direct contact between T-2 and pulmonary tissue, it followed that the early deaths observed after an aerosol exposure to T-2 in either suspension or solution could be due to a similar dose of T-2 reaching the pulmonary tissue, regardless of aerosol concentration. Experiments were designed, therefore, to quantitate and compare the actual deposition and retention of T-2 in the respiratory tract of mice exposed to aerosols of T-2 toxin either in suspension or solution. These studies were conducted with T-2 at an aerosol mass concentration that produced 100% very early deaths (i.e., < 10 hours) as well as at 24-hour LC₅₀ and LC₁₀ aerosol concentrations. With each aerosol mass concentration tested, total deposition (i.e., total body burden) of T-2 was greater for mice exposed to an aerosol of a T-2 solution than for mice exposed to an aerosol generated from a T-2 suspension (Creasia,

unpublished data). This was expected since it was necessary to expose mice to higher mass concentrations of the aerosol of T-2 in solution than the T-2 in suspension to achieve equal biological endpoints (i.e., LC₁₀₀, LC₅₀, LC_{LO}) for each aerosol. Moreover, at equal biological endpoints for each aerosol, the amount of T-2 retained in the lower respiratory tract immediately (less than 10 minutes) postexposure was, in fact, essentially equal in mice exposed to either T-2 aerosol. Thus, to establish a dose-response relationship for aerosols of T-2 in solution or in suspension, endpoints for mice such as early deaths, 24-hour LC₅₀, etc. are best correlated with the T-2 burden of the lower respiratory tract rather than with either aerosol mass concentration or total deposition of T-2 aerosol.

Essentially the same studies are now being completed with rats. Thus far, the data indicate that the rat is more susceptible to T-2 than the mouse (LC₅₀ for an aerosol of T-2 in solution in 0.020 ± 0.007 $\mu\text{g/L}$ for rat vs 0.080 ± 0.020 $\mu\text{g/L}$ for mice), but the qualitative response of the rat to inhaled T-2 appears to be similar to that of the mouse.

V. CHARACTERIZATION OF THE INHALATION TOXICITY OF T-2 TOXIN USING SWINE

A. The Swine Model

Swine were chosen (R.J.L.) as a model for human exposure to the toxin because: (1) they share many anatomic and physiological similarities with people,¹⁶⁻¹⁸ (2) 30-to 50-kg pigs have total lung volumes and respiratory rates similar to humans,^{19,20} (3) the pattern of lung development parallels that of the human,²¹⁻²² and (4) their microscopic and mesoscopic pulmonary anatomy is more like that of the human than that of other species commonly used in inhalation studies.²³⁻²⁵

B. Aerosol Generation and Characterization

Because pigs have elaborate nasal conchae which are efficient particle filters and the toxin is expensive, we elected to bypass the conchae and administer the toxin via an endotracheal tube, directly to the trachea and, hence, the lungs. The generation and administration system, as well as the methods used to expose swine to aerosols of T-2 toxin, have been described previously.²⁶ We regard this method as a model for human open-mouth breathing of aerosols.

The T-2 toxin used in these studies was produced from cultures of Fusarium sporotrichoides and was greater than 95% pure. Using a nebulizer, dry, filtered dilution air and absolute ethanol as a solvent, we were able to generate a dry aerosol as assessed by examination of the collected particles by light microscopy. The mass median aerodynamic diameter of the aerosolized particles, as calculated from the material collected by a 7-stage cascade impactor, was 1.5 μM with a geometric standard deviation of 2.4. The average mass concentration of the aerosols was 400 μg of toxin per liter of air.

C. Preliminary Studies

In an effort to define the exposure conditions which would produce mild to moderate clinical signs of T-2 toxicosis but not death, we exposed pigs to different doses of T-2 toxin by varying the duration of exposure and, therefore, the quantity of toxin nebulized. After dosing, the pigs typically exhibited chewing behavior and vomited. They then became anorectic (in spite of being held off feed for the previous 12 to 24 hours), would not

consume water, and were lethargic. Within about 4 hours, the skin and extremities (ears, nose, and limbs) were cyanotic and cold to the touch; capillary refill time was prolonged from the normal 2 to 5 seconds to 30 seconds or more. Within 6 hours, diarrhea occurred in some pigs, which persisted until recovery or death. With a sublethal exposure, the signs gradually subsided, and by 24 hours after exposure, the animals appeared clinically normal although feed intake was reduced. Animals exposed to a lethal amount of toxin became laterally recumbent and unable to rise. The heart rate increased; reaching 200 beats per minute or more; the expiratory rate increased; and the expiratory phase was prolonged. The animals died within 9 to 18 hours (retained dose approximately 3 mg/kg of T-2 toxin). The progression of clinical signs was largely consistent with those described for swine given the toxin intravascularly.²⁷⁻²⁹

Control animals exposed to the vapors of the ethanol vehicle did not appear to be clinically affected by the exposure. Immediately after dosing, they ate and drank when returned to their cages.

At the mass concentration of aerosol chosen to produce clinical signs of T-2 toxicosis but not deaths, it was necessary to expose the animals to the aerosol for as long as 45 minutes, thereby nebulizing as much as 8 mg of T-2 toxin per kg body weight. Deaths occurred with longer exposures resulting in the nebulization of 10 mg or more of the toxin per kg body weight. Estimates made with

the simultaneous nebulization of a short-lived isotope (^{99m}technetium) suggested that 20 to 30% of the toxin nebulized was actually retained by the pigs.

Gross lesions seen in swine that died as a result of these early exposures to T-2 toxin were similar to those seen in pigs given lethal intravascular doses of the toxin.²⁷⁻³¹ The gastric mucosa was diffusely dark red, particularly in the fundic portion, with a small amount of frank hemorrhage. Microscopically, extensive hemorrhage, congestion, edema, and fibrin thrombi were present in the gastric mucosa. The duodenal mucosa was slightly red, while those of the jejunum and ileum were dark red, especially along Peyer's patches. Microscopically, there was extensive necrosis of the surface and crypt epithelium and prominent lymphocyte necrosis in the lamina propria of the mucosa. These changes were most severe in the jejunum and ileum. The mucosa of the cecum and spiral colon was mottled red. The wall of the gallbladder was markedly edematous. Severe subcapsular and interstitial edema was seen in the pancreas, and the pancreatic parenchyma of the pigs that died was dark grey to pink. Vacuolization and necrosis were present in the exocrine portion of the pancreas.

In pigs that died, the heart musculature was soft, and severe subendocardial ecchymotic hemorrhage was present in the left ventricle. The meninges were markedly congested.

The lymph nodes (submandibular, cervical, bronchial, pancreatic, mesenteric, and inguinal) were mottled red and

edematous, especially in the subcapsular region. Histologically, marked lymphoid necrosis was seen in all of the lymphoid tissues.

D. Effects on Serum Biochemistry and Immunology

After the preliminary pigs, we initiated studies on the effects of inhaling sublethal doses of T-2 toxin on the hematology and serum biochemistry of swine.³¹ Nine- to 10-week-old, castrated, male, cross-bred, specific pathogen-free pigs were exposed to an aerosol of T-2 toxin for 45 to 61 minutes with 8 mg of T-2 toxin nebulized per kilogram of body weight and deposition of about 20 to 30%. Aerosol characteristics were as described previously. The T-2-dosed animals exhibited clinical signs consistent with T-2 toxicosis, such as altered peripheral circulation, lethargy, lateral recumbency, and anorexia but appeared clinically normal by 24 hours after dosing (though feed intake did not return to normal levels until several days after exposure).

The T-2 toxin-exposed pigs had marked elevations in serum alkaline phosphatase (ALP) and aspartate amino transferase (AST) activity at 24 hours, but these both declined and remained significantly lower than the control pig values from day 3 (ALP) or 10 (AST) through the end of the assessment period (2 weeks). There was also a gradual and significant decline in serum alanine amino transferase (ALT) activity after day 3. It is unlikely that the initial increase in serum ALP was a primary effect of liver necrosis because there was no concurrent rise in serum ALT activity.

There was a transient elevation in the blood urea nitrogen values of the T-2 treated pigs on day 1 after dosing. The serum

calcium concentrations of the pigs dosed with T-2 toxin were decreased at 24 hours after exposure. There were no treatment-related effects detected in serum lactate dehydrogenase activity or serum sodium, potassium, phosphorus, cholesterol, and glucose concentrations.

Exposure to the toxin resulted in transient alterations in blood leukocyte counts and differentials, as well as hemagglutination titers (sheep red blood cell) and lymphocytic blastogenic responses. These findings indicate that inhalation exposure to T-2 toxin can affect some aspects of humoral and cell-mediated immunity. (See the chapter entitled THE IMMUNOTOXICITY OF TRICHOTHECENE MYCOTOXINS).

E. Effects on Pulmonary Morphology and Immunology

In a subsequent study, Pang³¹ exposed 27- to 45-kg male castrated, crossbred, specific pathogen-free swine to aerosols of T-2 toxin for 42 to 65 minutes with 9 mg of the toxin nebulized per kilogram of body weight and retention of about 20 to 30%. The aerosol characteristics were similar to those described earlier. Litter mates were paired, with one being exposed to the toxin and the other to the ethanol vehicle. As a result of exposure to the toxin, one pig died after 8 hours and another, in a moribund state, was killed 10 hours after exposure. These two pigs comprised the 0.33-day treatment group. In addition, animals were killed at 1, 3, 5, and 7 days after exposure. Complete necropsies were performed on all pigs. The lung lobes of one side were perfused via the airways with a fixative and processed for morphological

examination. Cells were collected from the lobes of the other side via bronchoalveolar lavage.

At necropsy, small (2 to 3 mm diameter) dark red foci were randomly scattered in the lung lobes of some of the T-2 treated pigs on days 0.33 (2/2), 1 (2/5), 3 (1/5), and 7 (1/5).³¹ Histological examination of the T-2-exposed lung tissues from the 0.33-day pigs showed that they had a mild to moderate, patchy to diffuse, acute, interstitial pneumonia. The alveolar septa were slightly thickened due to congestion and infiltration of neutrophils and macrophages. There were variable amounts of an exudate consisting of neutrophils, macrophages, fibrin, and cellular debris in the alveolar spaces. Prominent lymphocyte necrosis was evident in the peribronchial and peribronchiolar lymphoid aggregates. Mild, scattered interstitial pneumonia was also seen in some of the T-2-treated pigs killed on days 1 (4/5), 3 (3/5), and 7 (2/5). The alveolar septa and spaces contained macrophages, neutrophils, and lymphocytes.

The alveolar macrophages recovered from the T-2 toxin exposed pigs at the earlier time points were less viable, had reduced phagocytic activity, and had impaired blastogenic responses (as above, for a more complete description of the immunologic effects, see the chapter entitled THE IMMUNOTOXICITY OF TRICHOHECENE MYCOTOXINS).

VI. SUMMARY

Intratracheally instilled T-2 toxin in saline suspension was over 20 times more toxic to mice and rats than was a T-2-ethanol or T-2-dimethyl sulfoxide solution. Preliminary conclusions indicate that the increased

toxicity was due to respiratory distress with concomitant alterations in blood pO_2 , pCO_2 , and pH. This hypothesis, however, did not stand up to further testing.

The toxicological responses of mice to aerosols generated from solutions or suspensions of T-2 toxin were similar, except that the animals were more severely affected when exposed to the suspensions. With the methods used, proportionately more of the toxin was retained in the lower respiratory tract when administered as a suspension than a solution.

Exposure of swine to aerosols of T-2 toxin resulted in clinical signs, death, and tissue lesions similar to those reported after intravascular administration of the toxin. In contrast to the findings described for rats and mice exposed for 10 minutes to the toxin, studies performed with swine (but with longer exposure periods) did not indicate that inhalation exposure to what appeared to be dry particles of the toxin was particularly more hazardous than intravascular exposure. The biochemical and immunological effects in swine caused by sublethal doses of aerosolized T-2 toxin appeared to be relatively short-lived. This may be related in part to the rapid plasma elimination of the parent compound^{27,28} and conversion of the toxin to less toxic metabolites.³³

Morphological changes in the lungs of swine were mild, though the edema, fibrin deposition, cellular infiltration, and debris in the alveolar spaces indicated that vascular and alveolar epithelial injury did occur. The lymphoid tissues, gastrointestinal tract, gallbladder, pancreas, and heart were severely affected in those animals that died as a result of the exposure. Under the exposure conditions used in these studies (RJL), it appears that the toxin reached the alveoli and, in

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spite of its crystalline state, was rapidly absorbed into the bloodstream and transported to other tissues in the body. The subsequent lesions were similar to those observed after intravascular administration of T-2 toxin at doses of 1.2 mg/kg or more.

The different responses, which seem to indicate that rodents are especially sensitive to respiratory tract exposure to particulate T-2 toxin, while swine are not, must be interpreted cautiously. Not only may species differences exist, but also the variation in time of exposure, vehicle, and manner of administration must be considered.

Many factors regarding the toxicity of inhaled T-2 need to be examined further. These include, but are not limited to, species differences in (1) respiratory and systemic immunotoxicology; (2) absorption and subsequent tissue and/or organ distribution, metabolism, and excretion; and (3) the results of thorough morphological examinations, including electron microscopy of respiratory tract tissue.

Finally, the reader is reminded that essentially all of the above research has been conducted in only two laboratories in a period of a little over a year. Although the basic conclusions are likely to be retained, much of the specific findings, such as LC₅₀'s, blood gas alterations, deposition data, etc. will be better defined as more studies are completed and a statistical baseline is achieved.

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